Nucl oside derivativ s and method for their production

The present invention concerns novel nucleoside derivatives as well as a method for their production.

Photolabile protective groups have already been described many times, particularly for the synthesis of oligomers. Particularly popular is their application to the synthesis of peptides and to the field of combinatory organic synthesis. The photolysis of protective groups is a relatively mild alternative to the traditional basic or acidic de-protection [methods] and is thus also particularly suitable for the synthesis of sensitive biomolecules. In this connection, in particular, numerous derivatives with ortho-nitrobenzyl functions have been successfully used, even for the the synthesis of oligonucleotides, e.g., on surfaces for production of oligonucleotide arrays (so-called biochips). Photo-cleavable protective groups should also be stable against basic and acidic reagents, which are applied in multi-step synthesis, and above all, they must not form highly reactive byproducts.

Nucleoside derivatives, which have been protected in the 5'-position with a derivativatized o-nitrobenzyloxycarbonyl or a 2-(o-nitrophenyl)ethoxycarbonyl function have been used almost exclusively up until now for the synthesis of oligonucleotides when photolabile protective groups are used. These functions can be effectively cleaved, for example, by irradiating with a Hg lamp, whereby

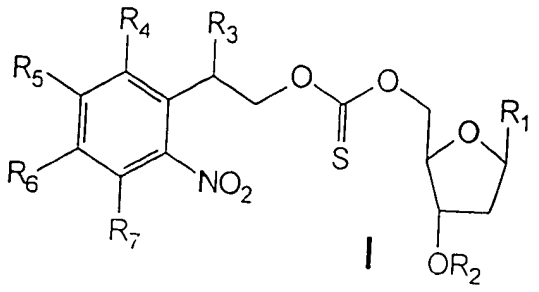
the emission line at 313 nm is a deciding factor. For example, o-nitrobenzyloxycarbonyl-protected nucleoside derivatives are also known for the commercial synthesis of oligomer arrays. Nucleoside building blocks with photolabile protective groups of the 2-(o-nitrophenyl)ethoxycarbonyl type are also known. The described protective groups, however, require relatively long irradiation times, for the most part of several minutes, for a complete cleavage of the nucleoside building block, so that secondary reactions must also be taken into consideration with sensitive biomolecules such as DNA.

Giegrich, H. et al. (Nucleosides and Nucleotides 17 (1998), pp. 1987-1996) describe the mentioned photolabile protective groups of the 2-(o-nitrophenyl)ethoxycarbonyl type. However, no indication is given that a thiocarbonyl function can also be used instead of the carbonyl function.

Photoreactive protective groups, which are of the 2(o-nitrophenyl)methoxy type are described in WO-A-94/10128. These compounds may also contain thiocarbonyl functions. The general formula, however, does not include compounds of the 2-(o-nitrophenyl)ethoxycarbonyl type.

The object of the present invention is thus to make available nucleoside derivatives, which can be easily photolysed.

The object is solved according to the invention in that nucleoside derivatives of the general formula I are created,

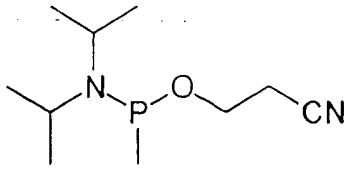


I

wherein

R_1 represents a nucleobase or a nucleobase provided with at least one protective group,

R_2 indicates an H atom or a diisopropylamino-(2-cyanoethoxy)phosphinyl group of formula IV



IV

R_3 is an H atom or an alkyl residue with up to 4 C atoms,

R_4 represents an H atom, a nitro group or an alkyl residue with up to 4 C atoms.

R_5 and R_6, independently of one another, represent an H atom, an alkyl residue with up to 4 C atoms, or an alkoxy residue with up to 4 C atoms or together represent a methylenedioxy group and

R_7 is an H atom or an alkyl residue with up to 4 C atoms.

According to the invention, it is preferred that R_1 is adenine, cytosine, guanine, thymine, uracil or hypoxanthine, which optionally bear a protective group.

In addition, it is preferred according to the invention that R_3 is an H atom, a methyl or an ethyl group.

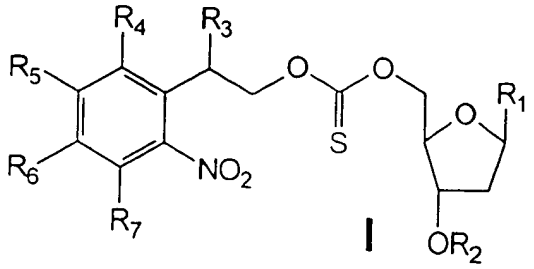
It is further preferred that

R_4 is an H atom, a nitro group or a methyl group.

It is additionally preferred that

R_5 and R_6, independently of one another, represent an H atom, a methyl, ethyl, methoxy or ethoxy group or together form a methylenedioxy group.

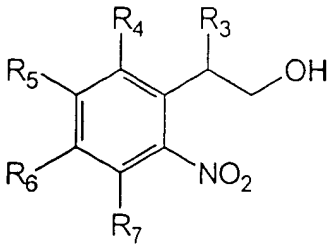
Another subject of the present invention is a method for the production of a nucleoside derivative of the general formula I



I

wherein the residues R_1, R_2, R_3, R_4, R_5, R_6 and R_7 have the above-indicated meaning,

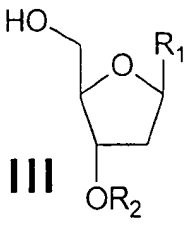
wherein a compound of the general formula II



II

wherein the residues R_3, R_4, R_5, R_6 and R_7 have the above-indicated meaning,

is reacted with thiophosgene in a way known in and of itself and the thus-obtained thiocarbonyl chlorides are reacted with a compound out the general formula III



III

wherein the residues R_1, and R_2 have the above-indicated meaning.

The present invention describes a novel type of photolabile protective groups on nucleoside derivatives (general formula I), which can be cleaved very efficiently.

The thiocarbonic acid esters corresponding to formula I can be produced in two steps, analogously to carbonic acid esters. First, a nitrobenzyl alcohol or a 2-phenylethanol derivative is reacted with thiophosgene to [form] the corresponding thiocarbonyl chloride and then coupled with the respective nucleoside building block. The nucleobase and the protective groups of the nucleoside building block have little influence on the synthesis. After introducing the photolabile protective group, the nucleoside building block can be converted into its phosphoramidite, so that it is accessible to established amidite chemistry, such as is conducted on commercial DNA synthesizers. The cleavage of the protective groups of the nucleoside derivatives according to formula I is carried out by means of irradiation with an Hg high-pressure lamp.

Another subject of the present invention is the use of the nucleoside derivatives according to the invention for the automatic synthesis of oligonucleotides. Here, automatic synthesizers and/or pipetting robots that are known in and of themselves are used in order to build up the desired oligonucleotides.

The subject of the present invention is also a kit for the automatic synthesis of oligonucleotides comprising at least one nucleoside derivative according to the invention, optionally together with other nucleoside derivatives according to the invention or already known, and reagents and adjuvants as well as solvents and operating instructions. The operating instructions may be present also in the form of a computer program for programming the automatic course of the individual synthesis steps. The desired oligonucleotides can easily be produced by means of automatic operating devices by means of this kit.

The following examples explain the invention:

Example 1:

(5'-(2-(2,6-dinitrophenyl)ethoxythiocarbonyl)thymidine

a) Preparation of 2-(2,6-dinitrophenyl)-1-ethanol

18.2 g of dinitrotoluene in 50 ml of absolute DMSO are loaded into a heated round-bottom flask and slowly mixed with a solution of 1.8 g of potassium tert-butylate in 20 ml of t-butanol. The initially slightly yellowish solution changes color to become intensely violet. The reaction mixture is first stirred at room temperature for 5 minutes and then at 70 °C for 10 minutes. It can be cooled and additionally stirred overnight at room temperature. For workup, it is neutralized with concentrated HCl and 300 ml of distilled water are added. NaCl is added to

the solution until the solution becomes saturated. The organic phase is separated and the aqueous phase is post-extracted several times with EtOAc. The combined organic phases are dried over $MgSO_4$. After filtering off the drying agent and extracting the solvent, the residue is taken up in rather hot EtOAc, overlayered with 100 ml of petroleum ether and left to crystallize overnight in the deep freezer. The petroleum ether is decanted, the residue is diluted with a few drops of toluene and applied onto a silica gel column. Toluene/EtOAc (5: 1) serves the mobile solvent, and if there is insufficient separating power during the elution, the polarity of the mobile solvent can be increased up to 3:1. The fractions containing product are combined and the solvent is distilled off. The reaction produced the pure product in a yield of 10.6 g (50%). Rf value (Silica 60; mobile solvent, toluene/EtOAc 8:1) = 0.36

b) Preparation of 2-(2,6-dinitrophenyl)ethoxythiocarbonyl chloride

400 µl of thiophosgene in 2.5 ml of absolute THF are loaded into a heated round-bottom flask flooded with argon and provided with a septum, cooled to 0°C and slowly mixed with a solution of 1 g of 2-(2,6-dinitrophenyl)ethanol in 7.5 ml of absolute THF. Stirring can be conducted for 20 minutes with ice cooling and then 1-3/4 h at room temperature and a control TLC (mobile solvent: chloroform) is conducted. The turbid solution is filtered through Celite and the filter cake is post-washed once more with THF. After the solvent has been extracted, a dark-

brown oily residue remains, which is further dried in vacuum and is reacted with 2'-deoxythimidine directly with the assumption of a 100% conversion.

c) Preparation of (5'-(2-(2,6-dinitrophenyl)ethoxythiocarbonyl)thymidine

583 mg of 2'-deoxythymidine is co-evaporated three times, each time with 1.5 ml of absolute pyridine, taken up in 5 ml of absolute pyridine and cooled to -50 °C by means of an isopropanol/N_2 bath. A solution of 1 g of thiocarbonyl chloride in absolute methylene chloride is slowly dripped in, and the temperature should not increase to above -20 °C. Additional stirring is conducted overnight at room temperature. A TLC control (mobile solvent dichloromethane/methanol = 100:5) shows a clear product spot, whereupon the reaction was terminated. For the workup, the content of the flask with 50 ml of dichloromethane is transferred to a feeding funnel and washed with 50 ml of distilled water. The aqueous phase is washed 3 times with 50 ml of methylene chloride each time, and the combined organic phases are dried over $MgSO_4$. The crude product concentrated to dryness is taken up in dichloromethane/methanol (2:1), and then applied to a silica gel column; first CH_2Cl_2/MeOH = 100:5 serves as the mobile solvent, and then the MeOH gradient can be increased to 100:7 toward the end of the elution. The reaction produced the product in a yield of 363 mg (30%) as a light brown powder. Rf value (Silica 60; mobile solvent, CH_2Cl_2/MeOH 9:1) = 0.88.

Example 2:

(5'-o-(2,2-nitrophenyl)propoxythiocarbonyl)thymidine

a) Preparation of 2-(2-nitrophenyl)propanol

3.02 g (2.69 ml) of 2-nitroethylbenzene and 600 mg of paraformaldehyde in 10 ml of DMSO are loaded into a heated round-bottom flask flooded with argon and mixed dropwise with a solution of 360 mg of potassium t-butylate in 4 ml of t-butanol. After finishing the addition, stirring is conducted for 15 minutes at room temperature and then heating is conducted for 1-3/4 h at 70°C. After the solution is cooled, it is transferred with EtOAc into a feeding funnel and washed with a saturated NaCl solution. The aqueous phase is post-washed twice with EtOAc, and the combined organic phases are dried over $MgSO_4$. The crude product is purified by column chromatography. Toluene/EtOAc (8:1) serves as the solvent, and the gradient can be increased to 6:1 toward the end of the chromatography. The product is eluted only at a very late time and is spread over a broad range of the column. The reaction supplied the pure product in a yield of 2.06 g (50%) as a reddish oil, Rf value (Silica60; mobile solvent, toluene/EtOAc 8: 1) = 0.28.

b) 2-(2-nitrophenyl)propoxythiocarbonyl chloride

754 ml of thiophosgene in 15 ml of absolute THF are loaded into a heated round-bottom flask flooded with argon and provided with a septum and mixed dropwise

with a solution of 1.5 g of alcohol and 1.536 g (1.115 ml) of triethylamine in 15 ml of THF with ice cooling. Stirring is conducted for one hour with ice cooling and for another hour at room temperature. The solution is filtered through Celite and the filter cake is post-washed with THF. The solvent is distilled on a rotary evaporator and the residue is stored at -20 °C. The reaction supplies the desired product as a light-brown oil in a yield of 2.09 g (97%).

c) Preparation of 5'-o-(2-(2-nitrophenyl)propoxythiocarbonyl)thymidine

1.48 g of 2'-deoxythymidine is co-evaporated three times with 15 ml of absolute pyridine each time, taken up in another 15 ml of pyridine and then cooled to -60°C by means of an isopropano/N_2 cold bath. A solution of 2.09 g of thiocarbonyl chloride in 20 ml of absolute dichloromethane is slowly sprayed [injected]; the solution is first stirred for 6 hours at -60°C and then overnight at room temperature. A DC control (mobile solvent of dichloromethane/methanol = 9:1) shows a clear product spot, whereupon the reaction was interrupted. For the workup, the content of the flask is transferred with 50 ml of dichloromethane into a feeding funnel, and washed with 50 ml of distilled water. The aqueous phase is post-washed three times, each time with 50 ml of dichloromethane, and the combined organic phases are dried over Na_2SO_4. For purification, the crude product is taken up in a small amount of dichloromethane/methanol and applied to a silica gel column. First, dichloromethane/methanol = 100:1 serves as the mobile solvent, and then the MeOH gradient can be increased to 100:4 toward

the end of the elution. The reaction produced the product as a light-brown foam in a yield of 937 mg (25%). Rf value (Silica 60; mobile solvent, CH_2Cl_2/MeOH 9:1) = 0.89.

Example 3: Cleavage of the protective groups

Decomposition of 5'-o-(2-(2-nitrophenyl)ethoxythiocarbonyl)thymidine by irradiation with UV light

In order to investigate the rate of decomposition of 5'-o-(2-(2-nitrophenyl)ethoxythiocarbonyl)thymidine, 1 mg of the compound is weighed out, dissolved in 1 ml of methanol and introduced into a quartz-glass cuvette (transmission in the wavelength range of 200 nm-2500 nm, layer thickness of 1 cm). The irradiation is produced by a mercury vapor lamp of the ORIEL Instruments company, Model 66057 (output of 250 W). In order to aviod an excess heating of the cuvette, an IR filter filled with water is connected in front. The cuvette is introduced into the beam path at a distance of approximately 20 cm from the lamp optics. After a half-minute interval each time, 10 µl of the solution are removed and analyzed by means of HPLC. The measurement values are shown in Figure 1.